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ANTIPARASITIC MACROLIDE CPDS. + WITH INSECTICIDAL, ACARICIDAL, ANTHELMINTIC AND ECTOPARASITICIDAL PROPERTIES

(AW) PARASITICIDAL

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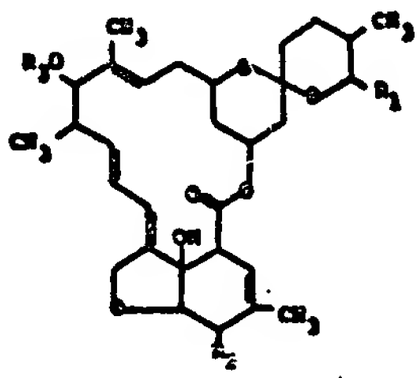
(54) Substituted hydrogenation products of C-076 compounds, derivatives thereof, their preparation and compositions for the treatment of parasitic infections containing the compounds.

(57) The invention provide compounds having the formula:



wherein R_1 is *iso*-propyl or *sec*-butyl; R_2 is methoxy, hydroxy or [illegible]noyloxy; and R_3 is hydrogen; lower alkanoyl; [illegible]yl; 4'-(lower alkanoyl)-α-L- oleandrosyl; [illegible]-α-L-oleandrosyl or 4"-(lower alkanoyl)-[illegible]-α-L-oleandrosyl. These compounds are [illegible] are produced by catalytic reduction [illegible] of the C-076 molecule. Further reaction [illegible] compounds is also possible. The compounds are [illegible] for their profound anthelmintic [illegible] and acaricidal activity. [illegible] including the described C-076 derivatives as [illegible] are also disclosed.

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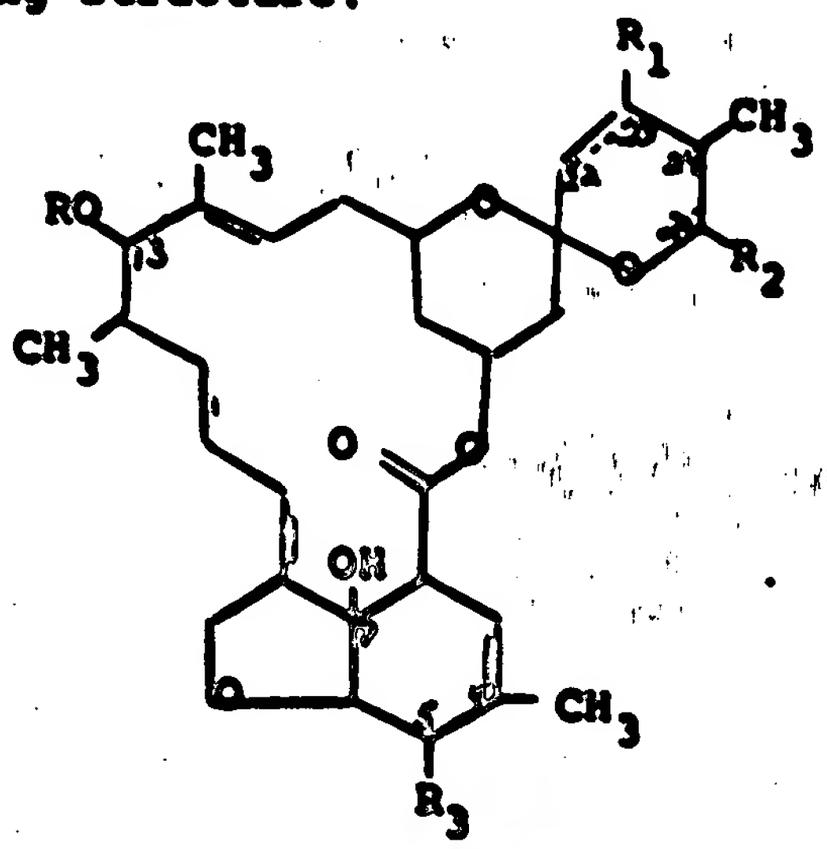
Croydon Printing Company Ltd.

Selective Hydrogenation Products of C-076 Compounds and Derivatives Thereof

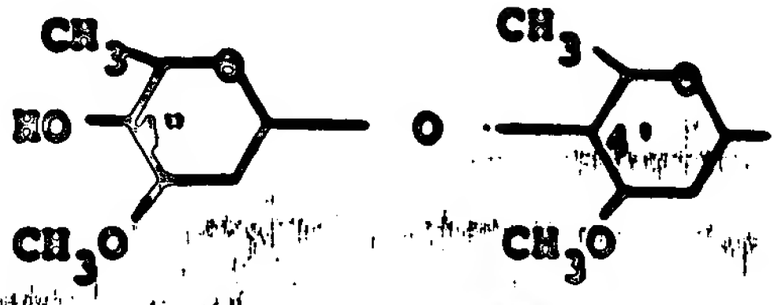
The term C-076 is used to describe a series of compounds isolated from the fermentation broth of a C-076 producing strain of Streptomyces avermitilis. The morphological characteristics of the culture are completely described below. The C-076 compounds are a series of macrolides, each of which is substituted at the 13-position with a 4-(a-L-oleandrosyl)-a-L-oleandrose group. The 1-series of C-076 compounds also has a 22,23-double bond, as well as several other double bonds. The selective reduction of the 22,23-double bond, without affecting the remaining double bonds is the subject matter of the present application. The C-076 compounds and the present derivatives thereof have a very high degree of anthelmintic and antiparasitic activity.

SUMMARY OF THE INVENTION

The C-076 series of compounds have the following structure:



wherein R is the 4'-(α-L-oleandrosyl)-α-L-oleandrose group of the structure:



and wherein the broken line indicates a single or a double bond; R_1 is hydroxy and is present only when said broken line indicates a single bond;

R_2 is iso-propyl or sec-butyl; and

R_3 is methoxy or hydroxy.

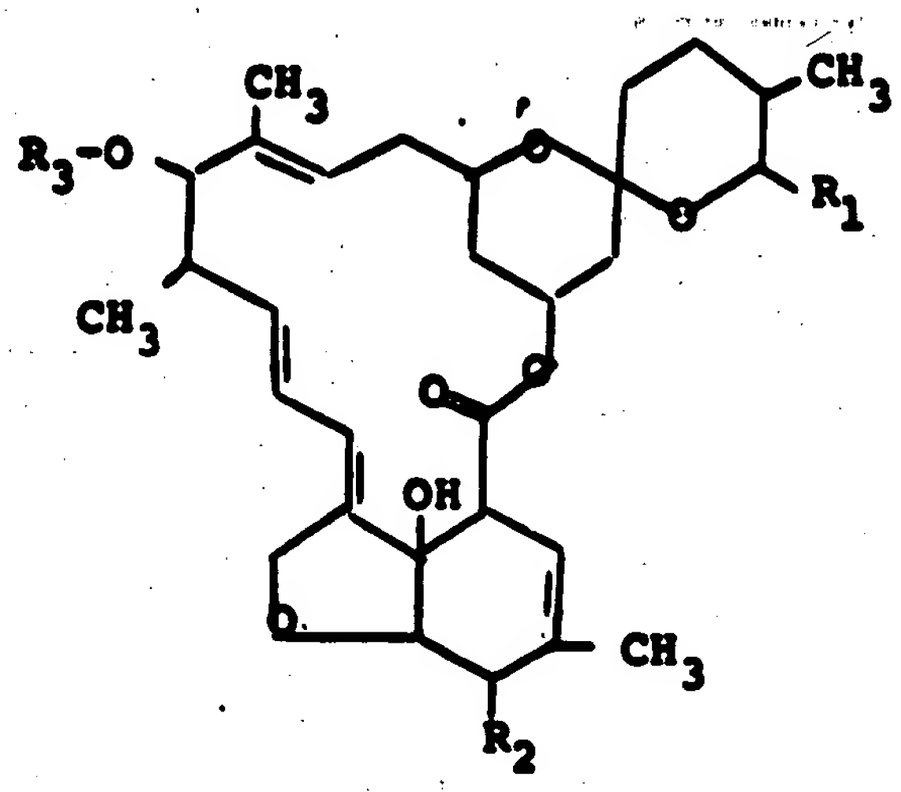
There are eight different C-076 compounds and they are given the designations A1a, A1b, A2a, A2b, B1a, B1b, B2a, B2b based upon the structure of the individual compounds.

In the foregoing structural formula, the individual C-076 compounds are as set forth below.

	R_1	R_2	R_3
A1a	Double bond	sec-butyl	$-OCH_3$
A1b	Double bond	iso-propyl	$-OCH_3$
A2a	-OH	sec-butyl	$-OCH_3$
A2b	-OH	iso-propyl	$-OCH_3$
B1a	Double bond	sec-butyl	-OH
B1b	Double bond	iso-propyl	-OH
B2a	-OH	sec-butyl	-OH
B2b	-OH	iso-propyl	-OH

The C-076 compounds with the 22,23-unsaturations are identified as the "1-series" and it is only these compounds which are reduced to prepare the instant derivatives. Either before or after the reduction of the 22,23-double bond further reactions may be carried out in which one or both of the a-L-oleandrose moieties are removed, or in which one or more of the available hydroxy groups are acylated.

Thus, the compounds of the instant invention have the following structural formula:



wherein

R_1 is iso-propyl or sec-butyl;

R_2 is methoxy, hydroxy or loweralkanoyloxy;

R_3 is hydrogen; loweralkanoyl; a-L-oleandrosyl; 4'-loweralkanoyl-a-L-oleandrosyl; 4'-(a-L-oleandrosyl)-a-L-oleandrosyl; 4"-loweralkanoyl-4'-(a-L-oleandrosyl)-a-L-oleandrosyl.

In the instant invention, the term "loweralkanoyl" is intended to include those alkanoyl groups of from 2 to 6 carbon atoms such as acetyl, propionyl, butyryl, pivaloyl and the like.

Preferred compounds of the instant invention are realized in the above structural formula when:

R_1 is iso-propyl or sec-butyl;

R_2 is methoxy or hydroxy; and

R_3 is hydrogen a-L-oleandrosyl or 4'-(a-L-oleandrosyl)-a-L-oleandrosyl.

Additional preferred compounds are realized when the "loweralkanoyl" group of R_3 is acetyl in the disaccharide, monosaccharide and aglycone compounds.

As is readily apparent from an analysis of the structure of the C-076 starting materials, there are five unsaturations in the 1-series of compounds. An object of the instant invention is to reduce the 22,23-double bond while not affecting the remaining four unsaturations or any other functional group present on the molecule. It is necessary to select a specific catalyst for the hydrogenation, one that will selectively hydrogenate the least hindered from among a series of unsaturations. The

preferred catalyst for such a selective hydrogenation procedure is one having the formula:

$$[(R_4)_3P]_3RhX$$

wherein R_4 is lower alkyl, phenyl, or lower alkyl substituted phenyl and X is a halogen.

In the preferred catalyst R_4 is phenyl and X is chlorine, that is the compound tris(triphenyl-phosphine)-rhodium (I) chloride, which is also known as Wilkinson's homogeneous catalyst.

The reaction is carried out using a catalytic amount of the catalyst. The amount of catalyst is not critical and from 0.05 to 0.5 moles of the catalyst for each mole of starting material have been successfully employed. Molar ratios in the range of 0.25 to 0.40 are preferred.

The hydrogenation is carried out in a hydrogen atmosphere which may be either at atmospheric pressure or up to about 4 atmospheres pressure in a standard laboratory hydrogenation apparatus. A solvent is normally employed to dissolve both the starting materials and the catalyst. Preferred solvents are hydrocarbon solvents such as benzene, toluene, petroleum ether and other alkane hydrocarbons. The reaction is complete when the calculated amount of hydrogen has been taken up by the reaction. This will generally require from about 1 to 48 hours. The reaction may be carried out at from room temperature to about 75°C, however, room temperature is preferred. The hydrogenation products are isolated

and purified by techniques known to those skilled in the art.

Other reactions may be carried out on the C-076 starting materials or on the hydrogenated products to prepare the compounds of this invention. While it is possible to complete all of the other reactions on the C-076 starting material and have the hydrogenation step as the final reaction, it is preferred to carry out the hydrogenation step first. Because the 22,23-double bond is somewhat susceptible to nucleophilic addition, reaction conditions for removing the sugar groups or acylating the hydroxy groups must be carefully controlled if the 22,23-double bond is present. If the 22,23-double bond is hydrogenated first, the subsequent sugar removal and acylation is rendered more facile.

Thus, the additional reactions which may be carried out to prepare the compounds of this invention are the selective removal of one or both of the a-L-oleandrosyl moieties or the selective acylation of the susceptible hydroxy groups.

The reaction conditions which are generally applicable to the preparation of both the monosaccharide and aglycone involve dissolving the C-076 compound or the hydrogenated C-076 compound in an aqueous acidic non-nucleophilic organic solvent, miscible with water, preferably dioxane, tetrohydrofuran, dimethoxyethane, dimethyl formamide, bis-2-methoxyethyl ether, and the like, in which the water concentration is from 0.1 to 20% by volume. Concentrated acid is added to the aqueous organic solvent to the extent of 0.01 to

10% by volume. The reaction mixture is generally stirred at about 20-40°C, preferably at room temperature, for from 6 to 24 hours. The lower concentrations of acid, from about 0.01 to 0.1% will predominately produce the monosaccharide under the above reaction conditions. Higher acid concentrations, from about 1 to 10% will predominantly produce the aglycone under the above reaction conditions. Intermediate acid concentrations will generally produce mixtures of monosaccharide and aglycone. The products are isolated, and mixtures are separated by techniques such as column, thin layer preparative and high pressure liquid chromatography, and other known techniques.

The acids which may be employed in the above process include mineral acids and organic acids such as sulfuric, hydrohalic, phosphoric, trifluoro-acetic, trifluoro methane sulfonic and the like. The hydrohalic acids are preferably hydrochloric or hydrobromic. The preferred acid in the above process is sulfuric acid.

A further procedure for the preparation of the monosaccharide or aglycone of the C-076 compounds or of the hydrogenated C-076 compounds utilizes a different solvent system for the monosaccharide and the aglycone. The procedure for the preparation of the monosaccharide uses 1% acid by volume in isopropanol at from 20-40°C, preferably room temperature, for from 6 to 24 hours. For the preparation of the aglycone, 1% acid, by volume, in methanol under the foregoing reaction conditions has been found to be appropriate.

When this procedure is employed on the starting material (the compounds with the 22,23-double bond) there is a possibility of nucleophilic addition to the double bond. If such occurs, chromatographic purification will remove the by-product in order to allow for further reactions.

The acids listed above are appropriate for this process, and again sulfuric acid is the preferred acid.

The above described compounds are isolated from the reaction mixture and mixtures of compounds are separated using techniques known to those skilled in this art, and in particular the chromatographic techniques described above.

The acylated compounds are prepared using acylation techniques in which the reaction conditions will vary, depending upon the reactivity of the hydroxy group being acylated. Where there is more than one hydroxy group to be acylated, different reaction conditions are employed to minimize the formation of mixtures.

The acylation reagents employed are generally the halide, preferably the chloride, of the above loweralkanoyl groups. That is the loweralkanoyl halide reagent is generally employed.

In addition, the acylation reagent could be in the form of the anhydride or of the halo formate. In the case of reactions carried out with the halide reagents, it is often advantageous to include in the reaction mixture a basic compound capable of

reacting with and neutralizing the hydrogen halide which is liberated during the course of the reaction. Tertiary amines are preferred such as triethylamine, pyridine, dimethylamino pyridine, diisopropyl ethylamine and the like. The basic compound is required in equimolar amounts relative to the numbered moles of hydrogen halide being liberated, however excess amounts, even using the basic compound as a solvent, are not detrimental.

In the case of the A1 compounds of C-076, or of the hydrogenated C-076 A1 compounds there is only a single hydroxy group, 4" hydroxy, which may be acylated. The formation of the monosaccharide or the aglycone still leaves only a single hydroxy group which may be acylated, that is the 4' or 13 hydroxy group.

In the case of the 4", 4' and 13 hydroxy groups of C-076 A1 compounds, the acylating reagent is dissolved in a suitable solvent, pyridine is preferred, and the acylating reagent added. The reaction is maintained at from 0°C to room temperature for from 4 to 24 hours. The product is isolated using known techniques.

The B1 compounds have 2 available hydroxy groups: at the 4"(4' or 13) and the 5-positions. However, the two hydroxy groups have similar reactivities. When the reaction of the acylating agent in pyridine is carried out at about room temperature for from 4 to 24 hours, the diacyl compound is recovered. When the reaction is carried out at 0°C a mixture of the 4"(4' or 13) and 5 monoacyl compounds are recovered. To recover individual compounds, the mixture is placed on a chromatographic column or a preparative layer chromatographic plate of

alumina or silica gel and the individual compounds are readily isolated. In addition, techniques such as high pressure liquid chromatography may be employed to separate mixtures of acylated compounds.

The acyl compounds thus prepared are isolated from the reaction mixture using techniques known to those skilled in this art.

The novel compounds of this invention have significant parasiticidal activity as anthelmintics, ectoparasiticides, insecticides and acaricides, in human and animal health and in agriculture.

The disease or group of diseases described generally as helminthiasis is due to infection of an animal host with parasitic worms known as helminths. Helminthiasis is a prevalent and serious economic problem in domesticated animals such as swine, sheep, horses, cattle, goats, dogs, cats and poultry. Among the helminths, the group of worms described as nematodes causes widespread and often times serious infection in various species of animals. The most common genera of nematodes infecting the animals referred to above are Haemonchus, Trichostrongylus, Ostertagia, Nematodirus, Cooperia, Ascaris, Bunostomum, Oesophagostomum, Chabertia, Trichuris, Strongylus, Trichonema, Dictyocaulus, Capillaria, Heterakis, Toxocara, Ascaridia, Oxyuris, Ancylostoma, Uncinaria, Toxascaris and Parascaris. Certain of these, such as Nematodirus, Cooperia, and Oesphagostomum attack primarily the intestinal tract while others, such as Haemonchus and Ostertagia, are more prevalent

in the stomach while still others such as Dictyocaulus are found in the lungs. Still other parasites may be located in other tissues and organs of the body such as the heart and blood vessels, subcutaneous and lymphatic tissue and the like. The parasitic infections known as helminthiases lead to anemia, malnutrition, weakness, weight loss, severe damage to the walls of the intestinal tract and other tissues and organs and, if left untreated, may result in death of the infected host. The hydrogenated C-076 compounds of this invention have unexpectedly high activity against these parasites, and in addition are also active against Dirofilaria in dogs, Nematospiroides, Syphacia, Aspiculuris in rodents, arthropod ectoparasites of animals and birds such as ticks, mites, lice, fleas, blowfly, in sheep Lucilia sp., biting insects and such migrating diperous larvae as Hypoderma sp. cattle, Gastrophilus in horses, and Cuterebra sp. in rodents.

The instant compounds are also useful against parasites which infect humans. The most common genera of parasites of the gastro-intestinal tract of man are Ancylostoma, Necator, Ascaris, Strongyloides, Trichinella, Capillaria, Trichuris, and Enterobius. Other medically important genera of parasites which are found in the blood or other tissues and organs outside the gastrointestinal tract are the filiarial worms such as Wuchereria, Brugia, Onchocerca and Loa, Dracunculus and extra intestinal stages of the intestinal worms Strongyloides and Trichinella. The compounds are also of value against arthropods

parasitising man, biting insects and other dipterous pests causing annoyance to man.

The compounds are also active against household pests such as the cockroach, Blatella sp., clothes moth, Tinola sp., carpet beetle, Attagenus sp., and the housefly Musca domestica.

The compounds are also useful against insect pests of stored grains such as Tribolium sp., Tenebrio sp. and of agricultural plants such as spider mites, (Tetranychus sp.), aphids, (Myzus sp.); against migratory orthopterans such as locusts and larval stages of insects living on plant tissue. The compounds are useful as a nematocide for the control of soil nematodes and plant parasites such as Meloidogyne sp. which may be of importance in agriculture.

These compounds may be administered orally in a unit dosage form such as a capsule, bolus or tablet, or as a liquid drench where used as an anthelmintic in animals. The drench is normally a solution, suspension or dispersion of the active ingredient usually in water together with a suspending agent such as bentonite and a wetting agent or like excipient. Generally, the drenches also contain an antifoaming agent. Drench formulations generally contains from about 0.001 to 0.5% by weight of the active compound. Preferred drench formulations may contain from 0.01 to 0.1% by weight. The capsules and boluses comprise the active ingredient admixed with a carrier vehicle such as starch, talc,

magnesium stearate, or di-calcium phosphate.

Where it is desired to administer the C-076 derivatives in a dry, solid unit dosage form, capsules, boluses or tablets containing the desired amount of active compound usually are employed. These dosage forms are prepared by intimately and uniformly mixing the active ingredient with suitable finely divided diluents, fillers, disintegrating agents and/or binders such as starch, lactose, talc, magnesium stearate, vegetable gums and the like. Such unit dosage formulations may be varied widely with respect to their total weight and content of the antiparasitic agent depending upon factors such as the type of host animal to be treated, the severity and type of infection and the weight of the host.

When the active compound is to be administered via an animal foodstuff, it is intimately dispersed in the feed or used as a top dressing or in the form of pellets which may then be added to the finished feed or optionally fed separately. Alternatively, the antiparasitic compounds of our invention may be administered to animals parenterally, for example, by intraruminal, intramuscular, intratracheal, or subcutaneous injection in which event the active ingredient is dissolved or dispersed in a liquid carrier vehicle. For parenteral administration, the active material is suitably admixed with an acceptable vehicle, preferably of the vegetable oil variety such as peanut oil, cotton seed oil and the like. Other parenteral

vehicles such as organic preparation using polketal, glycerol formal, and aqueous parenteral formulations are also used. The active monosaccharide or aglycone C-076 compound or compounds are dissolved or suspended in the parenteral formulation for administration; such formulations generally contain from 0.005 to 50 by weight of the active compound.

Although the antiparasitic agents of this invention find their primary use in the treatment and/or prevention of helminthiasis, they are also useful in the prevention and treatment of diseases caused by other parasites, for example, arthropod parasites such as ticks, lice, flies, mites and other biting insects in domesticated animals and poultry. They are also effective in treatment of parasitic diseases that occur in other animals including humans. The optimum amount to be employed for best results will, of course, depend upon the particular compound employed, the species of animal to be treated and the type and severity of parasitic infection or infestation. Generally good results are obtained with our novel compounds by the oral administration of from about 0.001 to 10 mg. per kg. of animal body weight, such total dose being given at one time or in divided doses over a relatively short period of time such as 1-5 days. With the preferred compounds of the invention, excellent control of such parasites is obtained in animals by administering from about 0.025 to 0.5 mg. per kg. of body weight in a single dose. Repeat treatments are given as required to combat re-infections

and are dependent upon the species of parasite and the husbandry techniques being employed. The techniques for administering those materials to animals are known to those skilled in the veterinary field.

When the compounds described herein are administered as a component of the feed of the animals, or dissolved or suspended in the drinking water, compositions are provided in which the active compound or compounds are intimately dispersed in an inert carrier or diluent. By inert carrier is meant one that will not react with the antiparasitic agent and one that may be administered safely to animals. Preferably, a carrier for feed administration is one that is, or may be, an ingredient of the animal ration.

Suitable compositions include feed premixes or supplements in which the active ingredient is present in relatively large amounts and which are suitable for direct feeding to the animal or for addition to the feed either directly or after an intermediate dilution or blending step. Typical carriers or diluents suitable for such compositions include, for example, distillers' dried grains, corn meal, citrus meal, fermentation residues, ground oyster shells, wheat shorts, molasses solubles, corn cob meal, edible bean mill feed, soya grits, crushed limestone and the like. The active hydrogenated C-076 compounds are intimately dispersed throughout the carrier by methods such as grinding, stirring, milling or tumbling. Compositions containing from about 0.005 to 2.0% by weight of the active compound are particularly suitable as feed premixes. Feed supplements, which are fed directly to the

animal, contain from about 0.0002 to 0.3% by weight of the active compounds.

Such supplements are added to the animal feed in an amount to give the finished feed the concentration of active compound desired for the treatment and control of parasitic diseases. Although the desired concentration of active compound will vary depending upon the factors previously mentioned as well as upon the particular C-076 derivative employed, the compounds of this invention are usually fed at concentrations of between 0.00001 to 0.002% in the feed in order to achieve the desired antiparasitic result.

In using the compounds of this invention, the individual hydrogenated C-076 components may be prepared and used in that form. Alternatively, mixtures of two or more of the individual hydrogenated C-076 components may be used, as well as mixtures of the parent C-076 compounds other C-076 compound or other active compounds not related to C-076 and the compounds of this invention.

In the isolation of the C-076 compounds, which serve as starting materials for the instant processes, from the fermentation broth, the various C-076 compounds will be found to have been prepared in unequal amounts. In particular an "a" series compound will be prepared in a higher proportion than the corresponding "b" series compound. The weight ratio of "a" series to the corresponding "b" series is about 75:25 to 99:1. The differences between the "a" series and "b" series is constant throughout the C-076 compounds and consists of a sec-butyl group and an iso-propyl group respectively at

the 25 position. This difference, of course, does not interfere with any of the instant reactions. In particular may not be necessary to separate the "b" components from the related "a" component. Separation of those closely related compounds is generally not practiced since the "b" compound is present only in a very small percent by weight, and the structural difference has negligible effect on the reaction processes and biological activities.

In particular it has been found that the starting materials for the compounds of this invention are very often prepared in a ratio of about 80% C-076 B1a or A1a and 20% C-076 B1b or A1b. Thus the preferred composition of this invention is one which contains about 80% of the "a" component and 20% of the "b" component.

The C-076 compounds of this invention are also useful in combatting agricultural pests that inflict damage upon crops while they are growing or while in storage. The compounds are applied using known techniques as sprays, dusts, emulsions and the like, to the growing or stored crops to effect protection from such agricultural pests.

The following examples are provided in order that this invention might be more fully understood; they are not to be construed as limitative of the invention.

The hydrogenated C-076 derivatives prepared in the following examples are generally isolated as amorphous solids and not as crystalline solids. They are thus characterized analytically using techniques such as mass spectrometry, nuclear magnetic resonance, and the like. Being amorphous, the compounds are not characterized by sharp melting points, however, the chromatographic and analytical methods employed indicate that the compounds are pure.

EXAMPLE 1

22,23-Dihydro C-076 Ala

52.0 Mg. of C-076 Ala and 14.4 mg. of tris (triphenylphosphine) rhodium (I) chloride are combined in 3.5 ml. of benzene and hydrogenated for 20 hours at room temperature under atmospheric pressure. The crude reaction mixture is chromatographed on a preparative layer chromatography plate eluting twice with 10% tetrahydrofuran in chloroform. The product is removed from the support using ethyl acetate which is evaporated to dryness and the residue analyzed with 300 MHz nuclear magnetic resonance and mass spectroscopy indicating the preparation of 22,23-dihydro C-076 Ala.

EXAMPLE 2

22,23-Dihydro C-076 Bla

The solution of 87.3 mg. of C-076 Bla in 6 ml. of benzene containing 25 mg. of tris (triphenylphosphine rhodium (I) chloride is hydrogenated for 4 hours at room temperature under 1 atmosphere of hydrogen pressure. Preparative layer chromatography on silica gel eluting with 20% tetrahydrofuran in chloroform recovers starting material. The sample is rehydrogenated following the above conditions for 19 hours. Preparative layer chromatography recovers 55 mg. of 22,23-dihydro C-076 Bla which is identified by mass spectrometry and 300 MHz nuclear magnetic resonance.

EXAMPLE 3

22,23-Dihydro C-076 B1a

A solution of 1.007 g. of C-076 B1a, 314 mg. of tris (triphenylphosphine) rhodium (I) chloride and 33 ml. of benzene is hydrogenated for 21 hours at room temperature under 1 atmosphere of hydrogen pressure. The solvent is removed in vacuo and the residue dissolved in a 1:1 mixture of methylene chloride and ethyl acetate and filtered. The filtrate is placed on a column of 60 g. of silica gel eluting with a 1:1 mixture of methylene chlorid and ethyl acetate taking 10 ml. fractions. Fractions 14-65 are combined and evaporated to dryness affording 1.118 g. of a solid material which is indicated by high pressure liquid chromatography to be a 60/40 mixture of the hydrogenated product and starting material. The mixture is rehydrogenated in 55 ml. of benzene adding 310 mg. of tris (triphenylphosphine) rhodium (I) chloride and stirring for 21 hours at room temperature under 1 atmosphere of hydrogen pressure. The solvent is removed in vacuo and the residue chromatographed on 80 g. of silica gel using 40:60 mixture of ethyl acetate and methylene chloride as eluant. 10 Ml. fractions are taken and the product appears in fractions 26-80. These fractions are combined and evaporated to dryness in vacuo affording a yellow oil. The oil is dissolved in benzene and lyophilized affording a pale yellow powder which is identified as 22,23-dihydro C-076 B1a by mass spectrometry and 300 MHz nuclear magnetic resonance. 0.976 G. of product is obtained.

EXAMPLE 4

22,23-Dihydro C-076 Ala Monosaccharide

11.2 Mg. of 22,23-dihydro C-076 Ala is dissolved in 1.1 ml. of 1% sulfuric acid in isopropanol and stirred for 20 hours at room temperature. The reaction mixture is diluted with chloroform to a volume of about 5.0 ml. and washed with saturated aqueous sodium bicarbonate solution and sodium chloride solution. The organic layer is dried over sodium sulfate and evaporated to dryness *in vacuo* affording an oil. The oil is placed on a silica gel preparative layer chromatography plate and eluted with 5% tetrahydrofuran in chloroform. The product is removed from the plate and lyophilized from benzene affording 5.2 mg. of a white powder which is identified by 300 MHz nuclear magnetic resonance and mass spectrometry as 22,23-dihydro C-076 Ala monosaccharide.

EXAMPLE 5

22,23-Dihydro C-076 Ala Aglycone

10.4 Mg. of 22,23-dihydro C-076 Ala is stirred for 20 hours in 1.1 ml. of 1% sulfuric acid in methanol at room temperature. The reaction mixture is treated as in Example 4 affording an oil which is purified by preparative layer chromatography on silica gel eluting with 5% tetrahydrofuran in chloroform. The product is removed from the chromatography plate and lyophilized from benzene affording 4.2 mg. of a white powder which 300 MHz nuclear magnetic resonance and mass spectrometry indicate to be 22,23-dihydro C-076 Ala aglycone.

EXAMPLE 6

22,23-Dihydro C-076 Bla Monosaccharide

395 Mg. of 22,23-dihydro C-076 Bla is added to a stirred solution of 50 ml. of 1% sulfuric acid in isopropanol and the solution is stirred for 14 hours at room temperature. The reaction mixture is treated as in Example 4 affording 0.404 g. of a foam after lyophilization from benzene. The foam is chromatographed on 6 preparative layer silica gel chromatography plates eluting twice with 4% tetrahydrofuran in chloroform. The monosaccharide with a Rf 0.15 is collected and washed from the silica gel with a total of 650 ml. of ethyl acetate. The combined washings are evaporated to dryness and the residue lyophilized from benzene to afford 0.2038 g. of 22,23-dihydro C-076 Bla monosaccharide which high pressure liquid chromatography indicates to be essentially pure.

EXAMPLE 7

22,23-Dihydro C-076 Bla Aglycone

9.7 Mg. of 22,23-dihydro C-076 Bla is stirred overnight in 1 ml. of a 1% sulfuric acid in methanol solution. The reaction mixture is treated as in Example 4 and the solid material treated with preparative layer chromatography on silica gel eluting with 10% tetrahydrofuran in chloroform. The oil recovered from the chromatography plate is lyophilized from benzene affording 4.7 mg. of a white powder which 300 MHz nuclear magnetic resonance and mass spectrometry indicate to be 22,23-dihydro C-076 Bla aglycone.

EXAMPLE 8

22,23-Dihydro C-076 Bla Aglycone

0.486 G. of 22,23-dihydro C-076 Bla is added to a stirred solution of 50 ml. of 1% sulfuric acid in methanol and the reaction mixture stirred for 13 hours at room temperature. The reaction mixture is diluted with 250 ml. of methylene chloride and washed with 50 ml. of saturated aqueous potassium bicarbonate and 50 ml. of water. The aqueous layer is washed twice with 20 ml. portions of methylene chloride and the combined organic phases are dried with saturated brine and sodium sulfate and evaporated to dryness in vacuo affording 0.480 g. of a pale yellow foam. The foam is dissolved in 4 ml. of methylene chloride and placed on 4 preparative layer chromatography silica gel plates and eluted 4 times with 4% tetrahydrofuran and chloroform. The product is recovered from the silica gel plates affording an oily residue which is lyophilized from benzene affording 255.8 mg. of a white solid. Traces of methyl oleandroside are indicated to be present in the solid material. The white solid is then lyophilized again from benzene and placed under high vacuum for 20 hours to remove the impurity affording 22,23-dihydro C-076 Bla aglycone.

EXAMPLE 9

4"-O-acetyl-22,23-Dihydro C-076 Ala

6.8 Mg. of 22,23-dihydro C-076 Ala is dissolved in 40 drops of anhydrous pyridine, chilled to 0°C and treated with 20 drops of acetic anhydride. The reaction mixture is allowed to warm to room temperature and stirred overnight. The reaction mixture is diluted with 5 ml. of ether and 6 ml. of water and the layers separated. The aqueous phase is washed twice with ether and the organic layers combined and back washed 3 times with water. The ether layer is dried over magnesium sulfate and evaporated to dryness in vacuo affording an oil. The oil is chromatographed on silica gel preparative layer chromatography plates eluting with 5% tetrahydrofuran in chloroform. The product is recovered from the plates and lyophilized from benzene affording 6.1 mg. of 4"-O-acetyl-22,23-dihydro C-076 Ala as determined by mass spectrometry at 300 MHz nuclear magnetic resonance.

EXAMPLE 10

4"-O-acetyl-22,23-Dihydro C-076 Bla and 4",5-di-O-acetyl 22,23-Dihydro C-076 Bla

18.6 Mg. of 22,23-dihydro C-076 Bla is dissolved in 63 drops (about 1 ml.) of dry pyridine and treated with 9 drops of acetic anhydride at 0°C. The reaction is stirred under nitrogen for 6 hours at 0°C. The mixture is then quenched with 5 ml. of water and extracted 3 times with 3 ml. portions of ether. The combined ether extracts are then washed 3 times with 3 ml. portions of water and dried

over magnesium sulfate and evaporated to dryness in vacuo. The oil is chromatographed on preparative layer silica gel chromatography plates eluting twice with 5% tetrahydrofuran in chloroform affording 5.8 mg. of 4"-O-acetyl-22,23-dihydro C-076 B1a and 5.8 mg. of 4",5-di-O-acetyl-22,23-dihydro C-076 B1a after lyophilization from benzene. The structures are confirmed by 300 MHz nuclear magnetic resonance and mass spectrometry.

EXAMPLE 11

22,23-Dihydro C-076 B1a

39 G. of C-076 B1a is dissolved in 1540 ml. of toluene and introduced into a 4 liter stirred autoclave. To this is added 3.9 g. of tris(triphenylphosphine)rhodium(I) chloride (Wilkinson's catalyst). A hydrogenation pressure of 40 psi. and a temperature of 40°C is maintained with stirring for 4 1/2 hours. At the end of this period liquid chromatographic analysis indicates 98% yield of dihydro C-076 B1a with 1.5% of tetrahydro C-076 B1a. The toluene is removed by evaporation in vacuo and the dark red gum is dissolved in ethanol at a rate of 4 ml. of ethanol per gram of product. Formamide at a rate of 10 ml. per gram of product is added and the solution heated on the steam bath to 40-50° while added water at a rate of 2 ml. per gram of product. After crystallization commences the heat is removed and the solution allowed to cool slowly with stirring overnight. The solid is filtered off and washed with a mixture 3 parts water and 1 part ethanol and dried in vacuo overnight. The solids are dissolved in 150 ml. of ethanol and warmed to 35-40°C on the steam bath. Water, 150 ml. is added slowly with stirring. When solution is complete at 35°C the heat is removed and the solution allowed to cool slowly overnight. The crystals are removed by

filtration and washed with 50% aqueous ethanol and dried in vacuo overnight affording 32.55 g. of 22,23-dihydro C-076 B1a with a m.p. of 155-157°C.

Based on taxonomic studies, the microorganisms capable of producing C-076 compounds are of a new species of the genus Streptomyces, which has been named Streptomyces avermitilis. One such culture, isolated from soil, is designated MA-4680 in the culture collection of Merck & Co. Inc., Rahway, New Jersey. A C-076-producing sample of this culture has been deposited in the permanent culture collection of the Fermentation Section of the Northern Utilization Research Branch, U.S. Department of Agriculture at Peoria, Illinois, and has been assigned the accession number NRRL 8165. A sample of NRRL 8165 has also been deposited, without restriction as to availability, in the permanent culture collection of the American Type Culture Collection at 12301 Parklawn Drive, Rockville, Maryland 20852, and has been assigned the accession number ATCC 31,267.

The morphological and cultural characteristics of Streptomyces avermitilis are set forth below:

Morphology: Sporophores form spirals on side branches on aerial mycelia. Spirals are compact but become more open as culture ages. Spores are in chains of more than 15 spores and are usually spherical to oval at 970 X magnification. Sporulation is observed on oatmeal agar, glycerol-asparagine agar, malt-starch agar and egg albumin agar. Spore surface is smooth as seen by electron microscopy.

Oatmeal agar

Vegetative growth:	Reverse - very dark brown
Aerial mycelium:	Powdery, brownish gray (4li)* mixed with white.
Soluble pigment:	Brown

Czapek Dox agar (sucrose nitrate agar)

Vegetative growth:	Poor, colorless
Aerial mycelium:	Scant, grayish
Soluble pigment:	Light grayish tan

Egg albumin agar

Vegetative growth:	Tan
Aerial mycelium:	Moderate, light grayish-yellow-brown (3ge)* mixed with white.
Soluble pigment:	Light yellowish tan

Glycerol asparagine agar

Vegetative growth:	Reverse - yellowish brown
Aerial mycelium:	Powdery, brownish gray (4li)* mixed with white.
Soluble pigment:	Light, yellowish brown

Inorganic salts-starch agar

Vegetative growth:	Reverse - grayish yellowish brown.
Aerial mycelium:	Powdery, light brownish gray (4ig)* edged with darker brownish gray (4li).*
Soluble pigment:	Light yellowish brown

Yeast extract-dextrose + salts agar

Vegetative growth:	Reverse - dark brown
Aerial mycelium:	Moderate, brownish white
Soluble pigment:	Brown

Yeast extract-malt extract agar

Vegetative growth:	Reverse - dark brown
Aerial mycelium:	Moderate, brownish white
Soluble pigment:	Brown

Peptone-iron-yeast extract agar

Vegetative growth:	Dark brown
Aerial Mycelium:	None
Soluble pigment:	Dark brown to black
Melanin:	Positive
H S production	Positive

Nutrient agar

Vegetative growth:	Tan
Aerial mycelium:	Sparse, grayish
Soluble pigment:	Light brown

Nutrient starch agar

Vegetative growth: Tan
Aerial mycelium: Sparse, grayish white
Soluble pigment: Light brown
Hydrolysis of starch: Good

Potato plug

Vegetative growth: Tan
Aerial mycelium: Brown mixed with grayish white
Soluble pigment: Grayish brown

Loeffler's Blood serum

Vegetative growth: Grayish tan
Aerial mycelium: None
Soluble pigment: Some browning of medium
Liquefaction: None

Nutrient tyrosine agar:

Vegetative growth: Reverse - dark brown to black
Aerial mycelium: Sparse, grayish
Soluble pigment: Dark brown
Decomposition of tyrosine: None

Carbon utilization

Pridham-Gottlieb basal medium + 1% carbon source; + = growth; no growth as compared to negative control (no carbon source).

Glucose	+
Arabinose	+
Cellulose	-
Fructose	+
Inositol	+
Lactose	+
Maltose	+
Mannitol	+
Mannose	+
Raffinose	+
Rhamnose	+
Sucrose	+
Xylose	+

Nutrient gelatin agar

Vegetative growth: Tan
Aerial mycelium: Sparse, grayish white
Soluble pigment: Light brown
Liquefaction of gelatin: Good

Gelatin stabs

Vegetative growth: Brown ring
Aerial mycelium: None
Soluble pigment: Greenish brown
Liquefaction of gelatin: Complete

Skim milk agar

Vegetative growth: Dark brown
Aerial mycelium: None
Soluble pigment: Dark brown
Hydrolysis of casein: Good

Litmus milk

Vegetative growth: Dark brown growth ring
Aerial mycelium: None
Color: Dark brown
Coagulation and/or peptonization: Complete peptonization; becoming alkaline (pH 8.1).

Skim milk

Vegetative growth: Dark brown growth ring
Aerial mycelium: None
Soluble pigment: Dark brown
Coagulation and/or peptonization: Complete peptonization; becoming alkaline (pH 8.0).

Temperature range: (Yeast extract-dextrose + salts agar)

28°C - Good vegetative growth and aerial mycelia
37°C - Good vegetative growth and aerial mycelia
50°C - No growth

Oxygen requirement: (Stab culture in yeast extract-dextrose + salts agar)

Aerobic

All readings taken after three weeks at 28°C unless noted otherwise. pH of all media approximately neutral (6.8 - 7.2)
Color number designations (*) taken from Color Harmony Manual, 1958, 4th Edition Container Corporation of America, Chicago, Illinois.

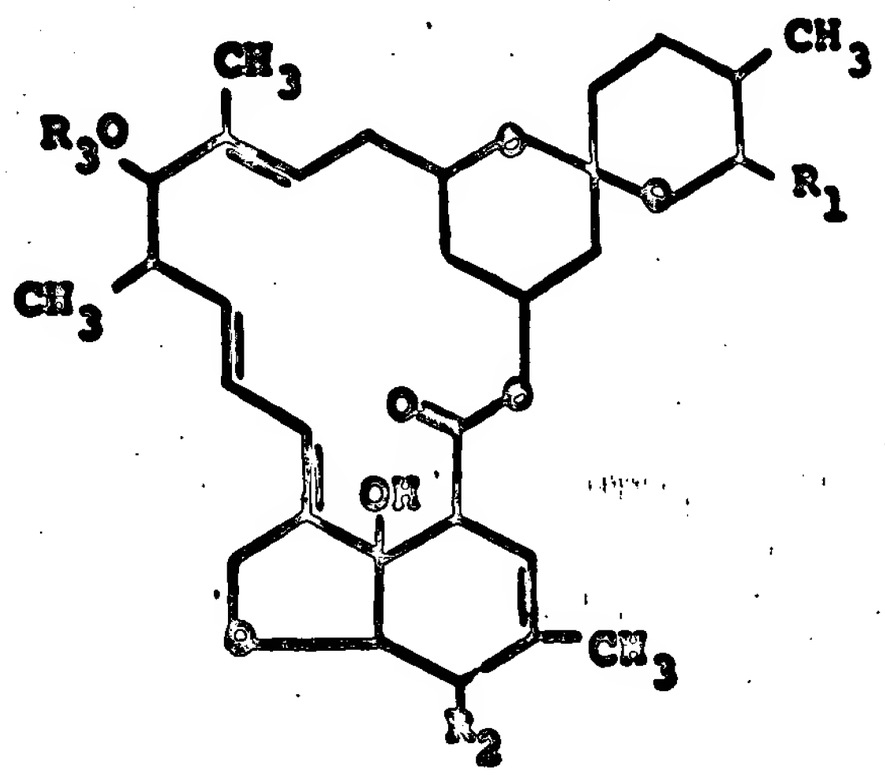
A careful comparison of the foregoing data with published descriptions including Bergey's Manual of Determinative Bacteriology (Eighth Edition) of known microorganisms reveals significant differences that indicate that the microorganism should be classified as a new species. On this basis, it was designed Streptomyces avermitilis.

Other organisms can also be used to produce C-076, e.g. mutants obtained by mutating agents such as X-ray irradiation, ultraviolet irradiation or nitrogen mustards.

A culture of one such organism was isolated after irradiating S. avermitilis with ultraviolet light. A lyophilized tube and a frozen vial of this culture have been deposited in the permanent culture collection of the American Type Culture Collection, and they have been assigned the accession numbers 31272 and 31271 respectively. Slightly higher fermentation yields of C-076 have been obtained using this frozen stock as inoculum.

CLAIMS

1. A compound having the formula :



in which R_1 is iso-propyl or sec-butyl; R_2 is methoxy, hydroxy or lower alkanoyloxy; and R_3 is hydrogen; lower alkanoyl; α-L-oleandrosyl; 4'-(lower alkanoyl)-α-L-oleandrosyl; 4'-(α-L-oleandrosyl)-α-L-oleandrosyl or 4"-(lower alkanoyl)-4'-(α-L-oleandrosyl)-α-L-oleandrosyl, where "lower alkanoyl" means an alkanoyl group containing from 2 to 6 carbon atoms.

2. A compound as claimed in Claim 1 in which R_1 is iso-propyl; R_2 is methoxy or hydroxy; and R_3 is hydrogen, α-L-

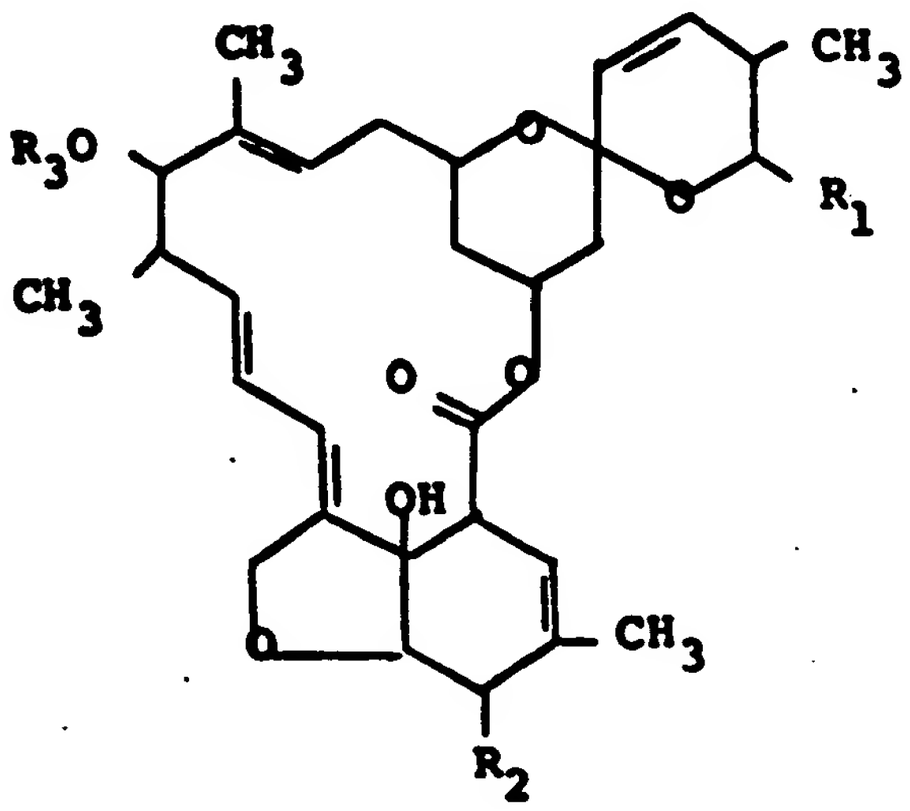
[illegible]yl or 4''-(α-L-oleandrosyl)-α-L-oleandrosyl.

3. A compound as claimed in Claim 2 in which R_1 is secondaryl and R_2 and R_3 are as defined in Claim 2.

4. 22,23-Dihydro-C-076 B1a.

5. 22,23-Dihydro C-076 B1a monosaccharide.

6. A method of preparing a compound as claimed in Claim 1 that comprises treating a compound having the formula :



in which R_1, R_2 and R_3 are as defined in Claim 1, with hydrogen in the presence of a catalytic amount of a compound having the formula $[(R_4)_3P]_3RhX$ in which R_4 is a lower alkyl, phenyl or (lower alkyl)-substituted phenyl radical and X is a halogen atom.

7. A compound as claimed in Claim 1 produced by a method as claimed in Claim 7.

8. The use as a parasiticide of a compound as claimed in Claim 1 or a mixture of two or more such compounds.

9. A composition for the treatment of parasitic infections that comprises an inert carrier and one or more compounds as claimed in Claim 1.

10. A composition as claimed in Claim 9 in which the active ingredient is a mixture of about 80% dihydrogenated C-076 B1a and, correspondingly, about 20% dihydrogenated C-076 B1b, as hereinbefore defined.

			C 07 D 493/22 A 01 N 9/28 C 07 H 17/08
P	DE - A - 2 717 040 (MERCK) • claim 10 • --	1-5	
A	DE - A - 2 329 486 (SANKYO) & US - A - 3 950 360 & US - A - 3 984 564 & US - A - 3 992 551 & US - A - 3 992 552 --		
A	Chemical Abstracts vol. 83, no. 15, 13 October 1975, Columbus, Ohio, USA, A. AOKI et al "Antibiotic B-41A, derivatives from Streptomyces as acaricides" page 163, column 1, abstract no. 127 530 s & JP - A - 7 529 742		

TECHNICAL FIELDS SEARCHED (Int. Cl.²)

A 01 N 9/28
C 07 H 17/08
C 07 D 493/22

CATEGORY OF CITED DOCUMENTS

X: particularly relevant
A: technological background
O: non-written disclosure
P: intermediate document
T: theory or principle underlying the invention
E: conflicting application
D: document cited in the application
L: citation for other reasons

&: member of the same patent family, corresponding document

1946 33746B 1210

X The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
Berlin	19-12-1978	FROELICH